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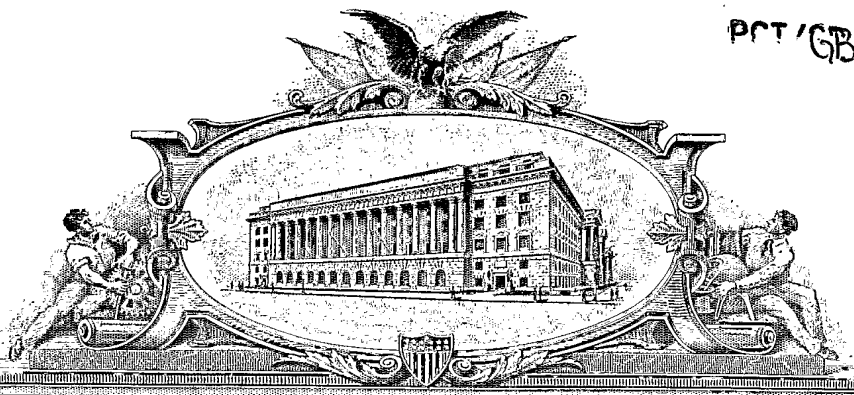

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FILING DATE: *March 25, 2004*

By Authority of the
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VIELKA BROWN
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PTO/SB/16 (08-03)
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

Express Mail Label No. EV146 601 389US **Date of Deposit:** March 25, 2004

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Paul David	Kemp	Romiley, United Kingdom
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☒ *Additional inventors are being named on the* Page 2 of 2 *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

WOUND HEALING COMPOSITION

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

☒ Customer Number 34139

OR

☐ Firm *or* Individual Name					
Address					
Address					
City		State		ZIP	
Country		Telephone		Fax	

ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification *Number of Pages* 25 ☐ CD(s), Number ____

☒ Drawing(s) *Number of Sheets* 02 ☒ Other (specify): Fee Transmittal for FY 2004

☐ Application Data Sheet. See 37 CFR 1.76

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☒ Applicant claims small entity status. See 37 CFR 1.27.

☐ A check or money order is enclosed to cover the filing fees

☒ The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 50-1275

☐ Payment by credit card. Form PTO-2038 is attached.

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Respectfully submitted,

SIGNATURE Doreen Yatko Trujillo Date 3/25/04

TYPED or PRINTED NAME Doreen Yatko Trujillo REGISTRATION NO. *(if appropriate)* 35,719

TELEPHONE (215) 665-5593 Docket Number: HARR0037-001

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PROVISIONAL APPLICATION COVER SHEET

Additional Page

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Docket Number **HARR0037-001**

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FEE TRANSMITTAL for FY 2004

Effective 10/01/2003. Patent fees are subject to annual revision.

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Application Number	Not Yet Assigned
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First Named Inventor	Paul D. Kemp et al.
Examiner Name	Not Yet Assigned
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Attorney Docket No.	HARR0037-001

METHOD OF PAYMENT *(check all that apply)*

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Deposit Account Name: Cozen O'Connor, P.C.

The Director is authorized to: *(check all that apply)*
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FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1001	770	2001	385	Utility filing fee	
1002	340	2002	170	Design filing fee	
1003	530	2003	265	Plant filing fee	
1004	770	2004	385	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	80.00

SUBTOTAL (1) ($) 80.00

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

			Extra Claims		Fee from below	=	Fee Paid
Total Claims	00	- 20 ** =	00	X		=	
Independent Claims	00	- 03 ** =	00	X		=	
Multiple Dependent						=	

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	86	2201	43	Independent claims in excess of 3
1203	290	2203	145	Multiple dependent claim, if not paid
1204	86	2204	43	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($)

**or number previously paid, if greater; For Reissues, see above*

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet.	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	420	2252	210	Extension for reply within second month	
1253	950	2253	475	Extension for reply within third month	
1254	1,480	2254	740	Extension for reply within fourth month	
1255	2,010	2255	1,005	Extension for reply within fifth month	
1401	330	2401	165	Notice of Appeal	
1402	330	2402	165	Filing a brief in support of an appeal	
1403	290	2403	145	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive – unavoidable	
1453	1,330	2453	665	Petition to revive – unintentional	
1501	1,330	2501	665	Utility issue fee (or reissue)	
1502	480	2502	240	Design issue fee	
1503	640	2503	320	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17 (q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	770	2809	385	Filing a submission after final rejection (37 CFR § 1.129(a))	
1810	770	2810	385	For each additional invention to be examined (37 CFR § 1.129(b))	
1801	770	2801	385	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	
Other fee (specify) ____					

*Reduced by Basic Filing Fee Paid SUBTOTAL (3) ($)

SUBMITTED BY

				Complete *(if applicable)*	
Name (Print/Type)	Doreen Yatko Trujillo	*Registration No. (Attorney/Agent)*	35,719	*Telephone*	(215) 665-5593
Signature	Doreen Yatko Trujillo			*Date*	March 25, 2004

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WOUND HEALING COMPOSITION

Field of the invention

The invention concerns compositions useful for assisting the process of wound healing, particularly chronic open lesions that are slow to heal. It conveniently delivers live cells with the capacity to initiate and encourage the healing process, in a biocompatible matrix.

Background to the invention

Healing of open wounds extending through the germinal epithelium in otherwise healthy tissue takes place by the process classically described as 'second intention', which, following initial haemostasis, involves a well-ordered sequence of inflammation, cellular infiltration, angiogenesis, granulation and re-epithelialisation. Where the wound has destroyed the germinal layer of the epithelium, collagen deposition by infiltrating fibroblasts and re-epithelialisation results in a degree of scarring, with incomplete restoration of function in terms of the flexibility and elasticity of the original dermis and failure to regenerate auxiliary structures such as hair follicles and sweat glands. Various approaches of autologous skin-grafting have long been used to close open wounds, minimise the risk of opportunistic infection, accelerate healing and minimise scarring.

However, a number of factors may adversely affect the rate and extent of such wound healing, in particular, poor blood supply. Poorly perfused tissue, often associated with impaired venous return and varicose veins, peripheral vascular disease or diabetes, often fails to heal satisfactorily, resulting in chronic ulcers, although the details of the pathogenesis are still unclear.

Current methods of skin grafting have significant limitations, not least the requirement for a suitable donor site from which grafts can be taken, especially where wounds are extensive (for example, burns). In addition, grafts have a low success rate where wound healing is compromised.

Chronic leg ulcers are a significant and growing problem world-wide. The introduction of compression therapy in combination with moist wound dressings has been the standard therapeutic management. More recently, tissue-engineering solutions have become available. Research into regenerative medicine has shown that human cells have substantial potential to heal and regenerate damaged tissue especially when primed by an environment that closely mimics the natural physiological condition being treated.

Much of the research has focused on the production of so-called 'tissue equivalents', which aim to provide a temporary functional replacement for missing tissue and accelerate healing. Such tissue equivalents may be dermal equivalents or total skin equivalents, the aim being to provide effective coverage of the wound as quickly as possible. The development and production of tissue equivalents usually involves the isolation of replacement skin cells, which are expanded and seeded onto / into a supporting structure such as a three-dimensional bio-resorbable matrix, or within a gel-based scaffold.

A variety of materials have been used as acellular protein matrices for wound healing applications.

These include synthetic polyesters (polyglycolic acid (PGA), polylactic acid (PLA), polyglactide (Dermagraft™, Smith & Nephew, described below), polydioxanone, polyhydroxyalkonoates and hyaluronic acid derivatives), hydrophilic polyurethanes (polyetherpolyester, polyethylene oxide and carboxymethylcellulose ethylene), and collagen-based scaffolds (cross-linked elastin collagen material (Matriderm™), cross-linked collagens manufactured from acid-soluble type I bovine collagen material (such as Vitaphore™). An alternative approach is to use an acellular derivative of allogeneic human dermis, a natural dermal matrix from which cells have been removed (such as Alloderm®, LifeCell Corporation). Some preparations use an organised, layered structure in order to more closely mimic the structure and function of the dermis. For instance, a preparation comprising an underlying layer of bovine collagen and shark glycosaminoglycans with an overlying layer of silicone is known (Integra®, Integra LifeSciences Corporation).

Other approaches have involved utilising fibrin sealants. Fibrin sealants (such as Tisseel™, Baxter; Beriplast™, Aventis; Quixil™, Omrix Biopharmaceuticals; Haemaseel™, Haemacure; and Crosseal™, Omrix) have been available for some time. All of these commercially available fibrin sealants are derived from cryoprecipitate from pooled plasma from virally screened allogeneic donors.

Such fibrin products rely on the natural polymerisation process that occurs during the physiological blood clotting cascade, in which a monomeric fibrin precursor, fibrinogen, is acted on by activated thrombin with the resultant production of polymeric fibrin. Fibrin forms the protein scaffold component of blood clots, to which platelets adhere.

More recently, fibrin has been recognised as a convenient and clinically acceptable cell carrier to be used in tissue engineering applications. Commercially available products that utilise fibrin sealants for cell delivery include Bioseed™ (Biotissue technologies), although several research groups make reference to the use of fibrin sealants for cell delivery

purposes results of which have shown some promise in burns (Brown *et al.* (1993) Am J Pathol 142: 273–283; Neidert *et al* (2001) 2001 Proceedings of the ASME Bioengineering Conference, Eds Kamm *et al*, Vol 50: 215–216; Tuan *et al* (1996) Exp Cell Res 223: 127–134; and US Patent application 200301654482)).

Exogenously applied dermal cells have been consistently shown to have beneficial effects on wound healing including shorter time to complete healing (Falanga and Sabolinski (1999) Wound Repair Regen 7: 210–207); delivery of active growth factors to the wound (Naughton *et al.* (1997) Artif Organs 21: 1203–1210); reduced potential for lesion recurrence (Gentzkow *et al* (1996) Diabetes Care 19: 350–354; and reduced pain (Muhart *et al* (1999) Arch Dermatol 135: 913–918).

As part of the normal healing response resident fibroblasts are required to undergo a series of phenotypic changes, migrating to the wound site, then proliferating, then synthesising and secreting extracellular matrix molecules. *In vivo*, a least a proportion then switch to a myofibroblastic phenotype in order to facilitate wound contraction (Clark (1993) Am J Med Sci 306: 42–48). *In vitro*, a series of phenotypically distinguishable mitotic and post-mitotic fibroblast populations have been described (Bayreuther *et al* (1988) Proc Natl Acad Sci USA 85: 5112–5116). This differentiation pathway appears to be controlled, at least in part, by interactions between fibroblasts and extracellular matrix (ECM) proteins present at the wound site. Growth factors and cytokines undoubtedly also exert an important influence, although their effects too, appear to be modulated by fibroblast exposure to particular ECM proteins (Eckes *et al* (1999) Springer Semin Immunopathol 21: 415–429; Xu and Clark (1996) J Cell Biol 132: 239–249). Among the ECM proteins that appear to have an important role in fibroblast differentiation are fibrinogen and fibrin. Fibroblasts specifically interact with fibrin and fibrinogen RGD motifs through $\alpha_v\beta_3$ integrin receptors (Gailit *et al* (1997) Exp Cell Res 232: 118–126) although the cellular response is complex and modulated by other factors. *In vitro* studies of the effect of fibrin glue on human periodontal ligament fibroblasts have suggested that fibrin appeared to slightly inhibit fibroblast proliferation (Fabris *et al* (1998) J Clin Periodontol 25: 11–14). The presence of a fibrin matrix has also been reported to increase the synthesis of collagen by entrapped fibroblasts (Neidert *et al*, 2001) The interaction between fibroblasts and ECM, in particular fibrin, and its influence on the activation and/or differentiation of fibroblasts is clearly of relevance when considering wound healing compositions comprising fibrin and live fibroblasts and attempting to optimise their therapeutic effects.

Fibroblasts also have a role in the remodelling of fibrin clots. As new extracellular matrix proteins such as collagen type I and III, fibronectin and vitronectin are laid down, the fibrin matrix is broken down, predominantly by the activation of the plasma-derived enzyme plasmin. This is regulated by the activation (or inhibition) of its proenzyme, plasminogen, by a variety of plasminogen activators and inhibitors. *In vivo*, a number of infiltrating cells, such as neutrophils and macrophages, secrete urokinase-type plasminogen activator (uPA), whilst endothelial cells are largely responsible for producing tissue plasminogen activator (tPA). However, it also clear that fibroblasts also secrete both uPA and plasminogen activator inhibitors, such as plasminogen activator inhibitor-1 (PA-1). The balance between these antagonistic mediators is crucial in controlling fibrin remodelling and scar formation (Tuan *et al*, 2003, Am J Pathol 162: 1579–1589). It is also clear that their expression is developmentally regulated, as well as being controlled by extracellular matrix components and local growth factors (Island *et al*, 1999, Wound Repair Regen 7: 467–476).

To facilitate movement through the cross-linked fibrin clot and a tight meshwork of extracellular matrix, a variety of fibroblast- and serum-derived enzymes cleave a path for migration. These include interstitial collagenase (matrix metalloproteinase-1, MMP-1), gelatinase (matrix metalloproteinase-2, MMP-2), stromelysin (matrix metalloproteinase-3, MMP-3) and the plasminogen activators (Grant GA, Eisen AZ, Marmer BL, Roswit WT, Goldberg GI (1987) J Biol Chem, 262: 5886-5889; Wilhelm SM, Collier IE, Kronberger A, Eisen AZ, Marmer BL, Grant BA, Bauer EA, and Goldberg GI (1987) Proc Natl Acad Sci USA, 84: 6725-6729; Stetler-Stevenson WG, Krutzsch HC, Wacher MP, Marguiles IMK, Liotta LA (1989) J Biol Chem, 264:1353-1356). Additionally, chemotactic factors, such as TGF-β and PDGF may also upregulate their production and secretion (Laiho M, Saksela O, Keski-Oja J (1986) Exp Cell Res, 164:399-407; Overall CM, Wrana JI and Sodek J (1989) J Biol Chem, 264: 1860-1869.).

Once migrating fibroblasts reached the wound, they gradually become secretory, whose major function is protein synthesis (Welch MP, Odland GF, Clark RAF (1990) J Cell Biol, 110:133-145). The previously retracted endoplasmic reticulum and Golgi apparatus becomes dispersed throughout the cytoplasm and a loose matrix is produced, which is mainly composed of fibronectin and type III collagen (Kurkinen M, Vaheri A, Roberts PJ, Stenman S (1980) Lab Invest, 43: 47-51; Grinnell F, Billingham RE, Burgess L (1981) 76:181-189). Ultimately, this profibrotic phenotype takes over, which is characterised by an abundance of rough endoplasmic reticulum and golgi apparatus, secreting newly synthesised collagen (Welch *et al.*, 1990) in response to highly expressed TGF-β.

Notwithstanding, TGF-β fails to upregulate further collagen deposition, once a matrix has been deposited (Clark RAF, Nielsen LD, Welch MP, McPherson JM (1995) J Cell Sci, 108: 1251-1261). It is also thought that IL-4 released by mast cells induces a modest increase in types I and III collagen together with fibronectin (Postlethwaite AE, Holness MA, Katai H, Raghow R (1992) J Clin Invest, 90:1479-1485). Mast cells furthermore produce tryptase (a serine esterase) in abundance, which has been shown to upregulate fibroblast proliferation (Ruoss SJ, Hartmann T, Caughey GH (1991) Clin Invest, 88: 493-499).

The stimuli responsible for fibroblast proliferation and matrix synthesis (TGF-α, TGF-β and PDGF) have been extensively investigated *in vitro* (Derynck R (1988) Cell, 54: 593-595; Ross RR and Raines EW (1990), Platelet-derived growth factor and cell proliferation, in: Growth Factors: From genes to clinical applications (VR Sara et al. eds.,) pp. 193-199, Raven Press, New York; Sporn MB and Roberts AM (1992) J Cell Biol, 119:1017-102) and their action was confirmed by *in vivo* manipulation of wounds as well (Sprugel KH, McPherson JM, Clowes AW, Ross R (1987) Am J Pathol, 129: 601-613; Pierce GF, Mustoe TA, Altrock B, Deuel TF and Thomas A (1991), Role of platelet-derived growth factor in wound healing, J Cell Biochem, 45:319-326). γ-interferon on the other hand was demonstrated to have a negative effect on the mitogenic and synthetic potential of fibroblasts *in vitro* and *in vivo* (Duncan MR and Berman B (1985) J Exp Med, 162: 516-527; Granstein RD, Murphy GF, Margolis RJ, Byrne MH and Amento EP (1987) J Clin Invest, 79: 1254-1258). In addition, the collagen matrix itself can suppress these activities (Grinnell, 1994; Clark *et al.*, 1995), whilst fibrin or fibronectin matrix has little or no suppressive effect (Clark *et al.*, 1995).

Interestingly, many fibroblasts undergo apoptosis (programmed cell death) in day-10 healing wounds (Williams GT (1991) Cell, 65: 1097-1098), thereby marking the transition from a fibroblast-rich granulation tissue to a scar tissue with reduced cell density.

Known combinations of protein matrices and live cells for wound healing applications may be summarised as follows.

A preparation comprising cryo-preserved primary human foreskin fibroblasts seeded onto a bioabsorbable glycolic-lactic acid polyester (polyglactide) scaffold (Dermagraft™, Smith & Nephew) is known for the treatment of ulcers (Naughton *et al*, 1997, US patent nos. 4,963,489,). The fibroblasts are allowed to proliferate in the scaffold, secreting extracellular matrix proteins and growth factors and cytokines. The mature preparation is packaged in

10% dimethylsulphoxide and bovine serum as a cryoprotectant to allow storage of the product by freezing prior to use. The disadvantages of this approach include difficulty in manipulating the product during application to the wound, and the necessity of storing and transporting the product at very low temperatures (-70°C) and use of careful thawing procedures in order to ensure viability of the cells (WO 87/06120).

Various combinations of collagen-based matrices and living cells are known. Apligraf® (Organogenesis Inc), is a bilayered structure comprising a lower ('dermal') layer of a bovine collagen scaffold supporting living human fibroblasts and a upper ('epidermal') layer comprising human keratinocytes on a collagen scaffold (Falanga and Sabolinski,1999, WO 99/63051). The preparation is supplied as a circular disk approximately 75 mm in diameter and 0.75 mm thick on an inert polycarbonate membrane. It is packaged individually for use and has 5-day shelf life. It is maintained in an agarose rich nutrient with a 10% CO_2/ air atmosphere and is shipped and stored at room temperature (20°C to 31°C; 68°F to 88°F). The removal of the product form the storage dish and polycarbonate membrane is involves teasing away the edge of the Apligraf using sterile forceps. Problems associated with this include excessive folding which can make accurate, close application of the preparation to the wound difficult and time consuming.

A similar product (Orcel™, Ortec International Inc) is described in US patent 6,039,760. This is also a bilayered structure of bovine collagen with fibroblasts and keratinocytes. The preparation is packaged between 2 non-adherent pieces of mesh, which are colour co-ordinated to distinguish between sides. The device is then packaged in a plastic tray containing media to maintain cell viability during storage and shipping, which is further packaged into pouches with chill packs to maintain a temperature of 11-19°C for 72 hours.

Other examples of tissue equivalents that attempted to reproduce a dermis-like arrangement of fibroblasts in a protein matrix supporting an overlying layer of keratinocytes are described in Meana *et al* (1998, Burns 24: 621–630).

Rama *et al* (2001, Transplantation 72: 1478–1485) describe a method of culturing autologous limbal stem cells on a fibrin gel substrate for grafting to the contralateral cornea.

US Patent application 20030165482 discloses a wound healing preparation (Allox™, Modex Therapeutiques SA) comprising growth-arrested allogeneic human fibroblasts applied to a wound in a viscous paste of fibrinogen (Tisseel®) to which thrombin has been added, so that

fibrinogen cleavage and fibrin polymerisation occur *in situ.* Alternatively, the separate liquid components are sprayed onto the wound, to set *in situ*, on mixing.

Summary of the invention

The invention provides an approach to treatment of chronic wounds based, not on providing an immediately functional tissue-equivalent, but on providing a means of delivering cells with the potential to accelerate the healing process together with a supportive, biologically-compatible protein matrix. Although developing a cultured dermal tissue equivalent comprising fibroblasts, extracellular matrix and overlying keratinocytes organised in to functional and anatomically relevant structures remains a worthwhile goal, so far this has proven elusive. However, for many situations, the present invention shows that such an approach may be unnecessarily complex and that a simpler solution, that of providing fibroblasts at the appropriate stage of maturity and exhibiting a particular phenotype embedded at appropriate cell densities in a fibrin (or other clottable protein) matrix, in a wound-healing composition for rapid, convenient and accurate application to wounds, is surprisingly effective. Although fibrin is a convenient and physiological extracellular matrix protein for use in a cell delivery product, the complex interaction between fibrin and fibroblasts also has important implications for ensuring that cells delivered to the wound surface have optimal wound healing potential. Optimally the cells must be in a synthetic phase of maturity, rather than a proliferative or senescent phase. Proliferation maybe useful to increase cell numbers, but delays the important synthesis of extracellular matrix proteins such as collagen types I and III, fibronectin and vitronectin. Cells that have become senescent do not contribute to wound healing and so serve little purpose in such a composition. An important aspect of the invention, therefore is use of young fibroblasts with a 'wound-healing phenotype (a phenotype that is either actively synthetic, or that can rapidly mature to such a phenotype), to encourage immediate wound healing, and the delivery of such cells to a wound in a matrix that is consistent with the maintenance of such a phenotype. Fibrin is such a matrix material, although other materials, such as agar, alginate, and polyvinyl alcohol (PVA) are also suitable.

Another important factor is the rate of fibrinolysis occurring within the composition. As described above, fibrinolysis is a normal part of the wound healing process, by which the fibrin matrix is gradually replaced by other extracellular matrix proteins. If, however, fibrinolysis occurs too early or too rapidly, the wound healing gel is broken down before useful collagen deposition has occurred. Fibroblast expression of pro-fibrinolytic factors

such as urokinase-type plasminogen activator is developmentally regulated and so the phenotype of fibroblasts included in the composition is of importance if premature fibrinolysis is to be avoided.

The fibroblast phenotype is therefore important to optimal wound healing. Human dermal fibroblasts in wound healing compositions in which they are embedded in collagen or in mature skin products have a different phenotype (for instance collagen gene expression will be very low as will fibrin and thrombin response genes). Preferably early gene expression will include elevated collagen gene expression and other genes involved in the early exposure of fibroblasts to a wound environment

Generating a gene expression profile allowing identification of appropriate early fibroblast phenotypes includes use of three different methods.

1. Quantitative PCR (TaqMan) is used to measure the amounts of particular genes being expressed by fibroblasts. The genes to be screened include: Gas6 (growth arrest-specific 6, also known as AXSF or AXLLG), collagen types I, III, and VI, PAI-1 (plasminogen activator inhibitor type-1), INSIG-1 (Insulin-induced gene 1), PLAU (plasminogen activator, urokinase), Collagen type VI, COX-2 (cyclo-oxygenase-2), PDGF (platelet-derived growth factor), vimentin, smooth muscle actin, apolipoprotein D, MMP-2 (matrix metalloprotease 2, gelatinase), GAPDH (glyceraldehyde-3-phosphate dehydrogenase), and RPL32 (ribosomal protein L32). Other informative genes include those encoding cytokines, metabolic genes, cytoskeletal genes, cell surface molecules and cell signalling molecules.

2. Differential display is a PCR-based method using non-specific primers, which produces a banding pattern when run on a gel that is unique to the sample of interest. This results in a "barcode" type pattern of gene expression. The advantage of this process is that it is fairly quick to perform and produces an easily recognisable pattern that is can be read off without numerical manipulation, or knowledge of the actual genes involved .

3. Microarray is a powerful technique for looking at global gene expression patterns. The advantage of this technique is that it looks at the gene expression of a large sample (~20,000) genes expressed by human cells. Therefore it is likely to capture all relevant genes.

It has been found that taking passaged human dermal fibroblasts, seeding them in a fibrin gel and then incubating them for 16–24 hours at 37° results in a phenotype that is particularly beneficial for use in wound healing applications. It has been observed that such cells are predominantly in a proliferative phase in culture (encouraged by low density seeding, avoiding contact inhibition). Under normal culture conditions, maturation to a wound-healing phenotype typically takes 2–3 days. However, incubation of fibroblasts in a fibrin gel shortens this maturation process, so that after 16–24 hours the cells are entering a wound-healing phenotype . Moreover, once they have reached this phase, they can preferably conveniently be stored at approximately 4°C for up to 19 days, and certainly 7–14 days before use without significant loss of viability or change of phenotype. This has significant practical advantages in that it provides not only an efficacious product comprising cells that are optimally suited for secretion of extracellular matrix with minimal inappropriate fibrinolysis, but also gives a relatively long shelf-life under commonly available standard refrigeration conditions. The ability to ship such products at approximately 4°C also considerably simplifies transportation. Maintaining a cold chain at 2°–8°C is considerably simpler and cheaper than shipping at -70°C, as is commonly required for live cells.

The product of the invention comprises a pre-cast fibrin gel in which passaged human dermal fibroblasts are seeded. The optimal ranges of cell density are believed to be from about 450 to about 2500 cells per mm^2, as measured per unit area of the cast gel. Within this range of cell densities, particular products have been developed using approximately 500 cells per mm^2 and approximately 1500 cells per mm^2.

One exemplary product uses a proprietary fibrin tissue sealant as described below in more detail in Example 1.

Accordingly, the invention provides a wound healing composition comprising living mammalian cells within a biological protein matrix characterised in that said matrix is pre-cast before topical application. Preferably said matrix comprises fibrin. Alternatively it may comprise an alternative clottable or gelling substance such as agar, alginate, PVA. Alternative suitable proteins include collagen, fibronectin and vitronectin.

Preferably said wound healing composition comprises living mammalian cells within a sterile, non-pyrogenic fibrin matrix characterised in that said fibrin matrix is pre-cast before topical application.

In a preferred embodiment said composition takes the form of a solid or semi-solid topical medicament. It is further preferred that the composition takes the form of a substantially disk-shaped cast gel, more preferably less than 8mm think, and most preferably less than 5mm thick.

In a preferred embodiment said cells are fibroblasts. Alternatively, they may be keratinocytes, stratum germinativum cells, or combinations or admixtures of such cells. More preferably, said fibroblasts are human dermal fibroblasts.

As discussed above, the optimal cell density is believed to be from about 450 to about 2500 cells per mm^2.

In one aspect the invention provides a composition containing 500 to 1500 cells per mm^2, preferably 450 to 550 cells per mm^2.

In an alternative aspect, the composition contains 750 to 2000 cells per mm^2, preferably 900 to 1700 cells per mm^2, most preferably 1450 to 1550 cells per mm^2

The concentration of fibrin can range from about 3 to about 20mg ml^{-1}.

In one alternative embodiment, the concentration of fibrin is in the range 3–5mg ml^{-1}. In a further alternative embodiment, the concentration of fibrin is in the range 5–20mg ml^{-1}, and is preferably in the range 7–12 mg ml^{-1}.

Also provided is method of manufacturing a wound healing composition, as described above, comprising: suspending living mammalian cells, in a solution comprising a protein monomer capable of polymerisation into an insoluble matrix, adding an agent capable of promoting such polymerisation and allowing polymerisation to occur in a mould such that the solid polymerised composition may be removed and packaged ready for topical administration to a patient. Preferably, said monomer is fibrinogen and said agent capable of promoting polymerisation is thrombin.

In a preferred embodiment, the method of the invention comprises use of a protease inhibitor, preferably, preferably a serine protease inhibitor, most preferably one selected from the list consisting of aprotinin, ε-aminocaproic acid, tranexamic acid.

Preferably, especially where the concentration of fibrin is in the range 7–12mg ml^{-1}, the protease inhibitor is aprotinin. Alternatively, especially where the concentration of fibrin is in the range 3–5mg ml^{-1}, the protease inhibitor is tranexamic acid.

In a further aspect, the invention provides the composition as herein described for use as a medicament, preferably for the treatment of a skin lesion, more preferably a skin lesion selected from the list consisting of venous ulcer, diabetic ulcer, pressure sore, burn, iatrogenic grafting wound.

Also provided is the use of a composition as herein described in the manufacture of a medicament to treat such a skin lesion and a process to manufacture a medicament for the treatment of such a skin lesion.

In a further aspect, the invention provides a package for a solid, or semi-solid, sterile, topical medicament comprising a flexible pouch consisting of two sheets of impermeable flexible material peripherally sealed to provide a means of containment, said pouch comprising one internal surface to which said medicament is preferentially adherent but to which the level of adhesion is less than that between the medicament and the bodily surface to be treated, such that the pouch may opened by parting said sheets and the medicament conveniently manipulated and directly to the surface to be treated without any requirement for the medicament to be directly touched by any other means before application. Preferably, the medicament comprises a wound healing composition comprising living cells, more preferably living mammalian cells within a sterile, non-pyrogenic fibrin matrix and most preferably the cells are human fibroblasts.

It is clear that such a package provides a convenient means of storage, delivery and application of any form of solid or, especially, semi-solid, materials, especially those intended for topical application to bodily surfaces. Preferably such materials are of a semi-solid or gel nature, such that physical manipulation is difficult. The preferential adherence of the material to an element of the packaging, with the ease of transfer thereafter to the skin or other bodily surface, provides a considerable advantage. In particular, such materials may be cut to the required size before application to the intended area. In the case of wound healing compositions as herein described, this is a particular advantage.

In a preferred embodiment, the package comprises metal foil, laminated or metalised plastic. In one preferred embodiment it comprises a transparent area allowing visual inspection of its contents.

Preferably, the internal surfaces of the package and its contents are sterile.

In a preferred embodiment one internal surface of the pouch is modified to increase the adherence of the medicament thereto. In one embodiment this comprises application of a coating to one internal surface. Preferably the coating is selected from the list consisting of: a polymer, a thermoplastic, a thermo-setting plastic, a protein, an amino acid, a carbohydrate.

Alternatively, one internal surface is modified by roughening to increase the adherence of the medicament thereto. As used herein, 'roughening' includes any physical modification of the surface intended to improve adherence, such as embossing, scratching, abrading or scuffing, or chemical roughening by means of etching, erosion, acid or alkali treatment. Other means of modifying the surface energy properties of the surface in order to improve or modulate the degree of adherence of the solid or semi-solid product are disclosed. Such means include coating one internal surface of the pouch. Preferably such a coating is selected from the list consisting of a polymer, thermoplastic, thermo-setting plastic, protein, amino acid or carbohydrate.

In one particularly preferred embodiment, the internal surface is modified by means of a discontinuous coating, in the form of raised areas or dots, having the effect of providing a roughened surface.

Also provided is a method of packaging a sterile, solid or semi-solid topical medicament comprising: placing said medicament in a sterile flexible pouch consisting of two sheets of impermeable flexible material incompletely peripherally sealed to provide a means of partial containment, said pouch comprising one internal surface to which said medicament is preferentially adherent but to which the level of adhesion is less than that between the medicament and the bodily surface to be treated; and completely peripherally sealing said pouch to provide a means of containment.

Preferably, the medicament comprises a wound healing composition comprising living cells, more preferably living mammalian cells within a sterile, non-pyrogenic fibrin matrix. Most preferably, the cells are human fibroblasts. One internal surface of the pouch is preferably modified to increase the adherence of the medicament thereto, as herein described.

In another aspect the invention provides a method of treating a patient suffering from a skin lesion or other appropriate condition, comprising the topical application of a composition as herein described.

The method of treatment comprises applying a solid or semi-solid topical medicament by means of flexible pouch composed of two sheets of impermeable flexible material sealed to provide a means of containment and in which the medicament is preferentially adherent to the internal surface contributed by one of said sheets; comprising opening the pouch by parting the sealed sheets and manipulating the medicament whilst adherent to one of the sheets and applying the medication to the surface to be treated without any requirement for the medicament to be directly touched by any other means.
Preferably, the medicament is a wound healing composition comprises living cells, more preferably mammalian cells within a sterile, non-pyrogenic fibrin matrix. Most preferably, the cells are human fibroblasts.

Preferably the skin lesion to be treated is an ulcer or a burn, most preferably selected from the list consisting of venous ulcer, diabetic ulcer, pressure sore, burn, iatrogenic grafting wound.

In another aspect, the invention provides a means of storing and transporting a medicament comprising live mammalian cells at 2°–8°C.

Detailed description of the invention

The invention will now be described in detail with reference to the figures, wherein:
Figure 1 summarises the process of manufacturing the wound healing composition.
Figure 2 shows the packaging, manipulation and application of the wound healing composition. **A**: shows the set gel preferentially adhering to the modified internal surface of one of the two metalised plastic sheets of which the pouch is comprised. **B**: shows the use of one of the sheets of the package to apply the gel of wound healing composition to skin. Note that sheet may used to support the gel while both are cut to the appropriate shape and size. **C**: shows the wound healing composition in place.

The invention relates to wound healing composition consisting of a topically applied viable cells of regenerative potential supported by a gel containing a fibrin matrix. Human dermal fibroblasts of a synthetic phenotype are particularly suitable for this purpose, although for

some applications keratinocytes or a combination of fibroblasts and keratinocytes may be more suitable.

The process of manufacturing the product is summarised in Figure 1. In principle, the product comprises two components, which are added together in order to cast a gel. The first component comprises a solution of fibrinogen together with one or more protease inhibitors to prevent unwanted proteolysis by protease contaminants and premature matrix breakdown by cells during storage. In particular, contaminants may include the naturally fibrinolytic enzyme plasmin, or its precursor plasminogen. Serine protease inhibitors such as aprotinin, ε-aminocaproic acid, or its analogue tranexamic acid, are frequently used in order to inhibit plasmin or prevent its activation. Added to this fibrinogen solution is a suspension of living cells in a suitable medium or buffer solution. The second component comprises a solution of thrombin (an enzyme that naturally acts upon fibrinogen), calcium ions (a required cofactor), and a medium suitable for the culture of living cells. A further clotting factor, Factor XIII, is also activated by thrombin in the presence of calcium ions and can be present in the second component. Activated Factor XIII promotes polymerisation of monomeric fibrin (cleaved from fibrinogen by thrombin) into a three-dimensional protein insoluble scaffold. In order to cast the gel, these two components are combined and poured into a pre-coated suitable mould whilst still liquid. Although commonly circular, clearly the gels may be cast into any shape. For some applications, other shapes may be more suitable. In particular, essentially rectangular or elliptical gels may be more convenient for larger wounds.

Enzymatic cleavage of fibrinogen into fibrin monomers and polymerisation of these monomers results in setting of the liquid into a semi-solid gel in which living cells are suspended. For many applications, this gel is then maintained for a period of about 24 hours under suitable conditions for cell growth, division and secretion of extracellular matrix proteins, and other proteins such as growth factors . Following maturation, the cast gels are removed from the casting moulds and placed directly into sterile packages. A small amount of medium is added to each package to maintain the product during storage and shipping, and the packages are sealed. During storage and shipping the packages are maintained at a temperature of 2°–8°C.

The advantages of such a product over the currently available alternatives include the following.

The use of a fibrin sealant as a scaffold allows convenient topical delivery of cells to the wound. The pre-cast gel allows convenient and accurate application of regenerative cells to

the wound surface with control of the distribution and density of cells applied. Manufacture and shipping of other tissue equivalents takes approximately 3 weeks for the matrix alone, whereas the product of the invention may be manufactured in 10 days, or even as little as 2 days if sufficient growing cells are available.

These factors combine to give cost advantages, so that manufacture and production is more cost effective than other commercially available products.

As described below, the product of the invention also features a unique flat pack system (adhesive backing) ensuring maintenance of product during shipping and "ease of use" of final product.

The precast gels can be shipped and stored for approximately 7–11 days at 2-8°C, whereas other available products are either frozen or shipped at room temperature.

Example 1 High protein concentration product ('Protoderm 500' and '1500')

One embodiment is designed to optimise both rapid manufacturing of the product and rapid wound healing by containing cells and protein components at relatively high concentrations.

Matrix

In this embodiment the matrix protein is fibrin, derived from a commercial fibrinogen product kit, Tisseel® (Baxter). When reconstituted this provides a convenient two component system to which cells may be added. The key components of the matrix are summarised in Table 1. It should be noted that Tisseel® also contains Factor XIII, as well as plasmafibronectin and plasminogen.

Table 1

Component	Final concentration in cellularised scaffolds
Matrix protein (fibrinogen)	7.5 - 11.5 mg/ml
Aprotinin	300 K IU/ml
Thrombin	25 IU/ml
Calcium chloride	4 mM

As will be clear to one of appropriate skill in the art, the exact concentrations of these components are not critical. Fibrinogen may be used in concentrations of the approximate range 7–20mg ml^{-1} for this application, thrombin in the range 5-50 IU/ml (in fact, trace levels

of contaminating thrombin will eventually lead to fibrin formation and gel setting without additional thrombin, but this is inconvenient and unpredictable), and calcium chloride in the range 2–20mM. Aprotinin is used to prevent unwanted fibrinolysis but, again, the exact concentration is not critical.

Cells

Human dermal fibroblasts were obtained by culture of cells derived from neonatal foreskin tissue. Under GMP conditions, fibroblastic cells were isolated by collagenase digestion and expanded by culture and serial passage according to routine laboratory practice to establish a master cell bank (MCB). The MCB was screened against a panel of human and animal-derived viruses, bacteria, mycoplasma and fungi, and for tumorigenicity by a GLP-accredited facility and determined to be free of contamination. Several working cell banks (WCB) were then established for manufacture of the product, rescreened and stocks of cells frozen according to standard procedures. For manufacture, cells are thawed, recovered, expanded for 7 days in culture, and resuspended in appropriate volumes and densities as required. The cells were used at passage 7. Although early passage cells are strongly preferred, the exact passage number is not critical. Preferably it less than 20, more preferably it is less than 15, most preferably less than 10.

It is also envisaged that for various patient-specific applications, autologous fibroblasts or other cells obtained from biopsies may be cultured and expanded for use.

The density of cells used in the product is expressed in terms of the number of cells per unit area of the cast gel, which is in the range 450–2500 cells mm^{-2}, more preferably 750–2000 cells mm^{-2}, further preferably 900–1700 cells mm^{-2}, and most preferably approximately 1500 cells mm^{-2}. In order to maximise the rate of healing, it may be desirable to use a cell density of approximately 1500 cells mm^{-2}. The cells are suspended in Liebovitz L-15 cell culture medium buffered and supplemented as shown in Table 2 before addition to the fibrinogen component. As will be clear to one of skill in the art, medium not intended for use in a CO_2-enriched atmosphere (commonly used in tissue culture incubators or sealed flasks) must be appropriately buffered by some other system. Such media, supplemented with, for instance, HEPES, are well-known in the art. Liebovitz L-15 medium relies on a phosphate buffering system. The medium was supplemented with sodium bicarbonate and dextrose, as shown.

For convenience and consistency a standard 'working cell suspension' of $1.5x10^6$ cells ml^{-1} is generally prepared.

Preparation of fibrin sealant

As outlined in Figure 1 and summarised below, Tisseel™ thrombin powder is reconstituted in a calcium chloride solution according to the manufacturer's directions.

Once dissolved, the Thrombin/$CaCl_2$ solution is further diluted with supplemented L-15 medium to obtain a 'Working Thrombin Solution' and refrigerated until further use for a minimum of 15 minutes. (Gels may also be manufactured with 'Working Thrombin Solution' at room temperature.) Freeze-dried fibrinogen is reconstituted with an aprotinin solution before being added to the working cell suspension in supplemented L-15 medium. Once reconstituted, the fibrinogen should be used within 4 hours, ideally within 1-2 hours.

Working thrombin solution (6.75 ml) contains:

Thrombin: 50IU/ml (or 337.5IU total)

Calcium chloride: 8umoles/ml (or 54 umoles total)

In supplemented L-15

(Total refers to the amount in 6.75mls)

Working fibrinogen and cell suspension mix (total volume 6.75ml):

Tisseel: 19mg/ml (or 128.25mg total)

Aprotinin: 600KIU/ml (or 4050KIU total)

Cells: $1.2x10^6$ cell/ml ($8.1x10^6$ cells total for P-1500);
$0.4x10^6$ cell/ml ($2.7x10^6$ cells total for P-500)

In supplemented L-15

(Total refers to the amount in 6.75mls)

Table 2 Medium

Components	Function	Concentration per ml
L-15 medium	Nutrient delivery to the cellular component of the product. Maintains cell viability and structure of the gel.	N/A (base medium)
Sodium Bicarbonate	Required for cell viability	202.5ug
Dextrose	Nutrient	4.5mg
Adenine	Base required for cell viability	24.4ug
L-Glutamine	Amino acid for cell viability	0.29mg
Ethanolamine	Phospholipid for cell metabolism	6.2ug
O-phosphoryl-ethanolamine	Phospholipid for cell metabolism	14.12ug
Hydrocortisone	Steroid required for cell metabolism	0.4mg
Human Recombinant Insulin	Essential hormone	5ug
Selenious acid	Trace substrate for metabolism	6.78ng
3,3',5-Triiodo-L-thyronine	Hormone	1.35ng
apo-Transferrin, bovine	Cofactor for iron metabolism	5ug
Gamma Irradiated Foetal Bovine serum	Nutrients	2%v/v

Casting the gels

The working thrombin solution (6.75ml) and Tisseel™ fibrinogen/cell suspension mixture (6.75ml) are combined by means of a Duplojet mixer unit and loaded into a suitable pre-coated casting container (conveniently a sterile Petri dish or similar) via a 16G needle or equivalent. It is necessary to pre-coat the casting dish with serum containing media or albumin to prevent the gel from adhering. The gel sets within a few minutes. The gel is then bathed in 20ml of medium (Table 2) and the casting dish covered with a lid. The set gel incubated at 37°C for 16–24 hours to allow maturation of the cells.

Packing and Storage

After maturation, the set gels are removed from their casting containers and placed into pre-irradiated, sterile foil pouches, stored within a sterile roto-seal bag. 10ml serum-free medium (as per Table 2, without the foetal bovine serum) is added to each pouch before sealing. The shelf life of the sealed units is approximately 11 days at 4°C.

Example 2 Low protein concentration product

For certain applications, it is possible to use lower protein concentrations. The chief advantage of this is reduction of production costs, since serum-derived proteins and many protease inhibitors, such as aprotinin, are expensive. In a preferred embodiment, the concentration of fibrin in the set product is reduced to less than 7 mg ml^{-1}. In practice, 3.0–4.0 mg ml^{-1} is found to be effective.

One important consideration is the effectiveness (as well as the cost) of aprotinin as the protease inhibitor in such 'low protein' products. In particular, *pro rata* dilution of commercial products results in aprotinin concentrations that are too low to be effective. A preferable solution is to use an alternative inhibitor, such as tranexamic acid. Not only is this a highly effective inhibitor of fibrinolysis, but has significant cost advantages.

Low protein concentration product:

Matrix

In this embodiment the matrix protein is fibrin, sourced from a commercial fibrin sealant, Tisseel™ using tranexamic acid instead of aprotinin. The key components of the matrix are summarised in Table 3. It should be noted that the same matrix composition could also be achieved using another commercially available fibrin sealant, Quixil. However the addition of exogenous tranexamic acid should be reduced as it already contains this inhibitor.

Table 3.

Component	Final concentration in cellularised scaffolds
Matrix protein (fibrinogen)	3.5mg/ml
Tranexamic acid	10mg/ml
Thrombin	25IU/ml
Calcium chloride	4mM

Freeze-dried Tisseel™ fibrinogen is reconstituted with supplemented L-15 medium solution before being added to the working cell suspension in supplemented L-15 medium. Once reconstituted, Tisseel™ fibrinogen should be used within 4 hours, ideally within 1-2 hours.

Tisseel™ thrombin powder is reconstituted in a calcium chloride solution according to the manufacturer's directions. Once dissolved, the thrombin/$CaCl_2$ solution is further diluted with supplemented L-15 medium containing tranexamic acid to obtain a working thrombin solution.

The cell density used is again in the range 450–2500 cells mm^{-2}. In order to minimise costs, it may be desirable to use a cell density of approximately 450–550 cells mm^{-2}. It should be noted, however, that protein concentration and cells density are independent variables. Lowering protein concentration is the major cost determinant, rather than cell density. However, being able to use fewer cells may have implications for the speed of production. In any case, high cell density / low protein concentration and low cell density / high protein concentration embodiments are envisaged and may be preferred in specific circumstances.

Example 3 Packaging, storage and delivery

A major factor contributing to the success of topical wound healing products is the ease of accurately applying them to the wound surface so that a close contact is established, without air bubbles or creases, under sterile operating conditions. Such products are, by their nature, fragile, and handling should be kept to a minimum. The product of the invention is packaged in such a way as to significantly assist and facilitate application. In addition, the product is shipped and stored chilled, rather than frozen, so that detailed thawing procedures are not required prior to use.

After setting and the 16–24 hour culture and maturation period, the individual gel discs are packaged by insertion into a flexible foil or metallised plastic pouch comprising two rectangular sheets, sealed along a substantial portion of three of their sides so as to form an open pocket. The inner surface of one of these sheets is modified so as to increase its adherence to the fibrin gel product. In a preferred embodiment the packaging used is an Oliver Products (Grand Rapids, Michigan USA) peelable foil pouch comprising one foil sheet and one sheet of laminated polyester/foil sheet with Q15 Adhesive dot pattern coating. Q15/48BF1 is a laminated lidding and pouching material for medical devices. The purpose

of this dot pattern adhesive coating is to improve the efficiency of the heat sealing process which is used to seal the edges of the sheets together. However, the adhesive and raised dot pattern prove highly effective in providing a surface to which the fibrin gel product preferentially adheres, as compared with the smooth, uncoated inner surface of the opposing sheet. It will be clear to one skilled in the art that other forms of coating and/or roughening of the surface of one of the internal surfaces of the pouch could be used to achieve the same effect. Similarly, any suitably durable, flexible, water and gas-impermeable sheet material might be used to manufacture such a pouch. All or part of the packaging might be transparent to allow visual inspection, for example, of the integrity of the gel or of the colour of a pH indicator dye in the cell culture medium, a small volume of which is inserted in the pouch, along with the product, before the pouch is sealed along its remaining open edge.

Thus sealed, the product has a shelf-life of at least 7–11 days, and preferably up to 19 days, at 2°–8°C.

For application, the pouch is peeled apart, under sterile conditions, leaving the fibrin gel product adhering to the treated inner surface of one of the sheets comprising the pouch. Using the sheet as a backing or means of support the gel is then applied to the surface of the wound, to which, in the absence of excessive exudation, it will preferentially adhere allowing it to be peeled away from the sheet. This means of application allows the gel to applied without wrinkling or incorporation of air bubbles, and with the minimum of manipulation. The edges of the product may be easily trimmed to fit the limits of the wound. Another advantage of delivering the product removeably adherent to the packaging in this way is that it allows the easy identification of the orientation of the product and facilitates oriented application. In the case of a homogenous wound-healing product, it is unimportant which way up the product is applied to the wound. However, where a multilayered product is involved, such as one with a fibroblast layer that is intended to be applied in contact with the wound surface and a keratinocyte layer that is intended to be oriented away from the wound surface, it may be difficult or impossible to establish the orientation of the product visually. Then the ability to deliver the product in such a way as makes incorrect application impossible without first removing the product from the packaging offers a significant advantage.

The foregoing examples are meant to illustrate the invention and do not limit it in any way. One of skill in the art will recognize modifications within the spirit and scope of the invention as indicated in the claims.

All references cited herein are hereby incorporated by reference.

What we claim is:

1. A wound healing composition comprising living mammalian cells within a sterile non-pyrogenic fibrin matrix characterised in that said fibrin matrix is pre-cast before topical application.

2. The composition of claim 1 wherein said matrix is 5mm or less thick

3. The composition of either of claim 1 or 2 wherein said cells are fibroblasts.

4. The composition of claim 3 wherein said fibroblasts are human dermal fibroblasts.

5. The composition of any one of claims 1 to 4 containing 450 to 2500 cells per mm^2.

6. The composition of claim 5 containing 450 to 550 cells per mm^2.

7. The composition of claim 7 containing 900 to 1700 cells per mm^2.

8. The composition of any of claims 1 to 7 wherein the concentration of fibrin is in the range 3–12mg ml^{-1}.

9. The composition of claim 8 wherein the concentration of fibrin is in the range 3–5mg ml^{-1}.

10. The composition of claim 8 wherein the concentration of fibrin is in the range 7–12mg ml^{-1}.

11. A method of manufacturing a wound healing composition comprising: suspending living mammalian cells in a solution comprising a protein monomer capable of polymerisation into an insoluble matrix, adding an agent capable of promoting such polymerisation and allowing polymerisation to occur in a mould such that the solid polymerised composition may be removed and packaged ready for topical administration to a patient.

12. The method of claim 11 wherein said monomer is fibrinogen.

13. The method of claim 12 wherein said agent capable of promoting polymerisation is thrombin.

14. The method of either of claims 11 to 13 wherein said cells are fibroblasts.

15. The method of claim 14 wherein said fibroblasts are human dermal fibroblasts.

16. The method of any one of claims 11 to 15 wherein the composition contains 450 to 2500 cells per mm^2.

17. The method of claim 16, wherein the composition contains 450 to 550 cells per mm^2.

18. The method of claim 16, wherein the composition contains 900 to 1700 cells per mm^2.

19. The method of any of claims 11 to 18 wherein the concentration of fibrin in the solid polymerised composition is in the range 3–12mg ml^{-1}.

20. The method of claim 19 wherein the concentration of fibrin is in the range 3–5mg ml^{-1}.

21. The method of claim 20 wherein the concentration of fibrin is in the range 7–12mg ml^{-1}.

22. The method of any of claims 11 to 21 further comprising use of a protease inhibitor.

23. The method of claim 22 wherein said protease inhibitor is selected from the list consisting of aprotinin and tranexamic acid, and combinations thereof .

24. The method of claim 23 wherein the protease inhibitor is tranexamic acid.

25. A composition according to any of claims 1 to 10 for use as a medicament.

26. A composition according to any of claims 1 to 10 for use as a medicament for the treatment of a skin lesion.

27. The composition of claim 26 wherein said skin lesion is selected from the list consisting of venous ulcer, diabetic ulcer, pressure sore, burn, iatrogenic grafting wound, and combinations thereof.

28. The use of a composition according to any of claims 1 to 10 in the manufacture of a medicament to treat a skin lesion.

29. The use of a composition according to claim 28 wherein the skin lesion is selected from the list consisting of venous ulcer, diabetic ulcer, pressure sore, burn, iatrogenic grafting wound, and combinations thereof.

30. A process to manufacture a medicament for the treatment of a skin lesion according to the method of any of claims 11 to 24.

31. The process of claim 30 wherein the skin lesion is selected from the list consisting of venous ulcer, diabetic ulcer, pressure sore, burn, iatrogenic grafting wound, and combinations thereof.

32. A method of treating a patient suffering from a skin lesion comprising the topical application of the composition on any of claims 1 to 10 to said skin lesion.

Application Serial No.: Not Yet Assigned
Title: WOUND HEALING COMPOSITION
Inventors: Paul D. Kemp, Györgyi Talas, Jennifer Sutherland and Margaret Batten
Docket No.: HARR0037-001 Filed: March 25, 2004
Atty: Doreen Yatko Trujillo Tel. No. (215) 665-5593

Figure 1

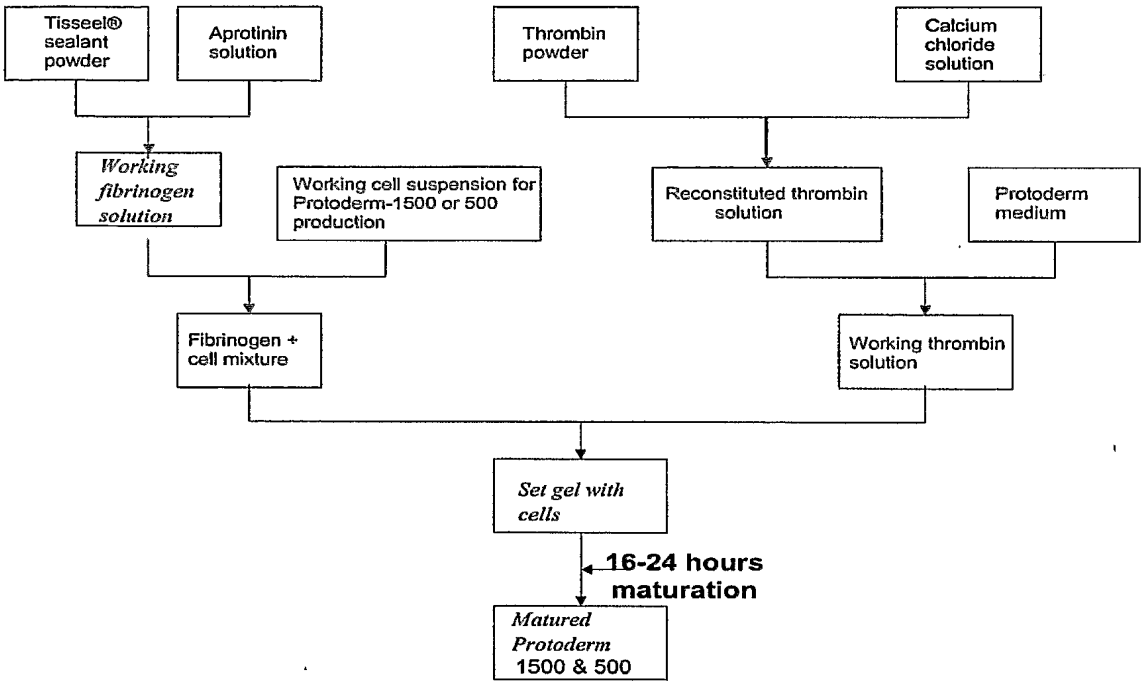

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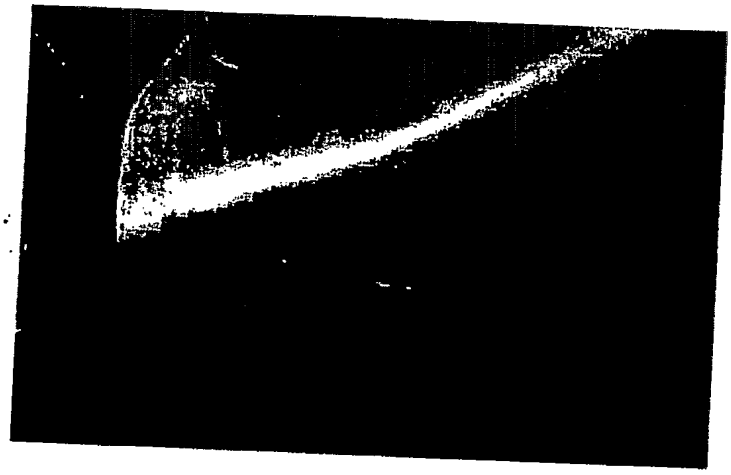
Figure 2

A

B



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